

AMENDMENTS TO THE CLAIMS

Applicant has submitted a new complete claim set showing marked up claims with insertions indicated by underlining and deletions indicated by strikeouts and/or double bracketing.

1. (Currently Amended) A method for labeling a target protein comprising contacting a fusion protein with a biotin analog, and allowing sufficient time for the biotin analog to be conjugated to the fusion protein via an acceptor peptide, in the presence of a biotin ligase mutant, wherein the fusion protein is a fusion of the target protein and the acceptor peptide, and wherein the biotin ligase mutant is a mutant of SEQ ID NO: 1 and comprises one or more amino acid substitutions selected from the group consisting of T90G, T90V, T90A, N91S, C107G, Q112M, G115A, Y132G, Y132A, S134G, V189G and I207S.
2. (Original) The method of claim 1, wherein the biotin analog comprises an aliphatic carboxylic acid tail.
3. (Withdrawn and Original) The method of claim 1, wherein the biotin analog comprises a substitution at a trans-ureido nitrogen (N) of biotin.
4. (Original) The method of claim 1, wherein the biotin analog is selected from the group consisting of an N-ketone biotin analog, a ketone biotin analog, an N-azide biotin analog, an azide biotin analog, an N-acyl azide biotin analog, an NBD-GABA biotin analog, a 1,2-diamine biotin analog, an N-alkyne biotin analog and a tetrathiol biotin analog.
- 5.-29. (Cancelled)
30. (Original) The method of claim 1, wherein the target protein is a cell surface protein.
31. (Original) The method of claim 1, wherein the fusion protein is in a cell.

32. (Original) The method of claim 31, wherein the cell expresses the biotin ligase mutant.
33. (Original) The method of claim 31, wherein the cell is a eukaryotic cell.
34. (Withdrawn and Original) The method of claim 31, wherein the cell is a bacterial cell.
35. (Original) The method of claim 33, wherein the eukaryotic cell is a mammalian cell, a Drosophila cell, a Zebrafish cell, a Xenopus cell, a yeast cell or a C. elegans cell.
36. (Withdrawn and Original) The method of claim 1, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 4.
37. (Original) The method of claim 1, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 5.
38. (Original) The method of claim 1, wherein the acceptor peptide is N- or C- terminally fused to the target protein.
- 39.-43. (Cancelled)
44. (Withdrawn and Currently Amended) The method of claim [[43]] 1, wherein the biotin analog is N-ketone biotin analog.
45. (Withdrawn and Currently Amended) The method of claim [[43]] 1, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 6.
- 46.-47. (Cancelled)

48. (Withdrawn and Currently Amended) The method of claim [[47]] 1, wherein the biotin ligase mutant comprises amino acid substitutions of T90G and N91S.

49. (Withdrawn and Original) The method of claim 48, wherein the biotin analog is N-alkyne biotin analog.

50. (Withdrawn and Original) The method of claim 48, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 7.

51.-52. (Cancelled)

53. (Original) The method of claim 1, wherein the method is performed in a cell free environment.

54. (Original) The method of claim 1, wherein the method is performed in a cell.

55. (Original) The method of claim 1, wherein the method is performed in a subject.

56. (Original) The method of claim 1, wherein the acceptor peptide is fused to the target protein via a cleavable bond or linker.

57-161. (Cancelled)